

1590612_1.TXT
SEQUENCE LISTING

<110> Collins, Peter L.
Murphy, Brian R.
Whitehead, Stephen S.

<120> PRODUCTION OF ATTENUATED CHIMERIC RESPIRATORY SYNCYTIAL VIRUS
VACCINES FROM CLONED NUCLEOTIDE SEQUENCES

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<141> 2003-11-25

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<151> 1999-04-13

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<151> 1997-07-15

<150> US 60/047,634
<151> 1997-05-23

<150> US 60/046,141
<151> 1997-05-09

<150> US 60/021,773
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| acaatttaac cataaccatt tggataacca ccagcggtta ttaaataata tattttgatga | 8340 |
| aattcattgg acacctaata acttattaga tgccactcaa caatttctcc aacatcttaa | 8400 |
| catccctgaa gatatatata caatatatat attagtgtca taatgcttgg ccataacgat | 8460 |
| tctatatcat ccaaccataa aactatctta ataagggtat gggacaaaat ggatcccat | 8520 |
| attaatggaa actctgctaa tgtgtatcta actgatagtt atttaaaagg tgttatctct | 8580 |
| ttttcagaat gtaatgcttt agggaggtac ctttttaacg gcccttatct caaaaatgat | 8640 |
| taccaacact taattagtag acaaagtcca ctactagagc atatgaatct taaaaaacta | 8700 |
| actataacac agtcattaat atctagatat cataaagggt aactgaaatt agaagaacca | 8760 |
| acttatttcc agtcattact tatgacatat aaaagcatgt cctcgtctga acaaatgtct | 8820 |
| acaactaact tacttaaaaa aataatacga agagctatag aaataagtga tgtaaagggtg | 8880 |
| tacgccatct tgaataaact aggactaaag gaaaaggaca gagttaagcc caacaataat | 8940 |
| tcagggtgatg aaaactcagt acttacaact ataattaaag atgatatact ttcggctgtg | 9000 |
| gaaagcaatc aatcatatac aaattcagac aaaaatcact cagtaaatca aaatatcact | 9060 |
| atcaaaacaa cactcttgaa aaaattgatg tgttcaatgc aacatcctcc atcatgggtta | 9120 |
| atacactggt tcaatttata tacaaaaatta aataacatat taacacaata tcgatcaaat | 9180 |
| gaggtaaaaa gtcatgggtt tatattaata gataatcaaa ctttaagtgg ttttcagttt | 9240 |
| attttaaatc aatatgggtg tatcgtttat cataaaggac tcaaaaaaat cacaactact | 9300 |
| acttacaatc aatttttaac atggaaagac atcagcctta gcagattaaa tgtttgctta | 9360 |
| attacttgga taagtaattg ttggaatata ttaaaaaaa gcttagggct gagatgtgga | 9420 |
| ttcaataatg ttgtgttatc acaattattt ctttatggag attgtatact gaaattattt | 9480 |
| cataatgaag gcttctacat aataaaagaa gttagagggt ttattatgct ttttaattcta | 9540 |
| aacataacag aagaagatca atttaggaaa cgattttata atagcatgct aaataacatc | 9600 |
| acagatgcag ctattaaggc tcaaaagaac ctactatcaa gggatgttca cactttatta | 9660 |
| gacaagacag tgcctgataa taticataat ggtaaatgga taatcctatt aagtaaattt | 9720 |
| cttaaatgga ttaagcttgc aggtgataat aatctcaata atttgagtga gctatatttt | 9780 |
| ctcttcagaa tctttggaca tccaatggtt gatgaaagac aagcaatgga tgctgtaaga | 9840 |

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| attaactgta | atgaaactaa | gttctactta | ttaagtagtc | taagtacggt | aagaggtgct | 9900 |
| ttcatttata | gaatcataaa | aggggttgta | aatacttaca | acagatggcc | cactttaagg | 9960 |
| aatgtctattg | tcctacctct | aagatgggta | aactattata | aacttaatac | ttatccatct | 10020 |
| ctacttgaaa | tcacagaaaa | tgatttgatt | attttatcag | gattgcggtt | ctatcgtgaa | 10080 |
| tttcatctgc | ctaaaaaagt | ggatcttgaa | atgataataa | atgacaaagc | catttcacct | 10140 |
| ccaaaagatc | taatattggac | tagttttcct | agaaattaca | tgccatcaca | tatacaaaat | 10200 |
| tatatagaac | atgaaaagtt | gaagttctct | gaaagcgaca | gatcaagaag | agtactagag | 10260 |
| tattacttga | gagataataa | attcaatgaa | tcgatcttat | acaattgtgt | agtcaatcaa | 10320 |
| agctatctca | acaactctaa | tcacgtggta | tcactaactg | gtaaagaaag | agagctcagt | 10380 |
| gtaggtagaa | tgtttgctat | gcaaccaggt | atgtttaggc | aaatccaaat | cttagcagag | 10440 |
| aaaatgatag | ccgaaaatat | tttacaattc | ttccctgaga | gtttgacaag | atatggtgat | 10500 |
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| aatgataact | acaacaatta | tatcagtaaa | tgttctatca | ttacagatct | tagcaaattc | 10620 |
| aatcaagcat | ttagatatga | aacatcatgt | atctgcagtg | atgtattaga | tgaactgcat | 10680 |
| ggagtacaat | ctctgttctc | ttggttgcat | ttaacaatac | ctctgtgcac | aataatatgt | 10740 |
| acatatagac | atgcacctcc | tttcataaag | gatcatgttg | ttaatcttaa | tgaagttgat | 10800 |
| gaacaaagtg | gattatacag | atatcatatg | ggtgggtattg | agggtcggtg | tcaaaaactg | 10860 |
| tggaccattg | aagctatatc | attattagat | ctaatacttc | tcaaggggaa | attctctatc | 10920 |
| acagctctga | taaatgggtga | taatcagtc | attgatataa | gtaaaccagt | tagacttata | 10980 |
| gagggtcaga | cccattgctca | agcagattat | ttgttagcat | taaatagcct | ttaattgcta | 11040 |
| tataaagagt | atgcaggtat | aggccataag | cttaagggaa | cagagaccta | tatatcccga | 11100 |
| gatatgcagt | tcattgagcaa | aacaatccag | cacaatggag | tgactatcc | agccagtatc | 11160 |
| aaaaaagtcc | tgagagtagg | tccattggata | aatacaatac | ttgatgattt | taaagttagt | 11220 |
| ttagaatcta | taggtagctt | aacacaggag | ttagaataca | gaggggaaag | cttattatgc | 11280 |
| agtttaatat | ttaggaacat | ttggtttatc | aatcaaattg | ctttgcaact | ccgaaatcat | 11340 |
| gcattatgta | acaataagct | atatttagat | atattgaaag | tattaaaaca | cttaaaaact | 11400 |
| ttttttaatc | ttgatagtat | cgatatggcg | ttatcattgt | atatgaattt | gcctatgctg | 11460 |
| tttgggtggtg | gtgatcctaa | tttgttatat | cgaagctttt | ataggagaac | tccagacttc | 11520 |
| cttacagaag | ctatagtaca | ttcagtggtt | gtgttgagct | attatactgg | tcacgattta | 11580 |
| caagataagc | tccaggatct | tccagatgat | agactgaaca | aattcttgac | atgtgtcatc | 11640 |
| acattcgata | aaaatcccaa | tgccgagttt | gtaacattga | tgagggatcc | acaggcggtta | 11700 |
| gggtctgaaa | ggcaagctaa | aattactagt | gagattaata | gatttagcagt | aacagaagtc | 11760 |

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| | | | | | | |
|------------|-------------|------------|------------|------------|-------------|-------|
| ttaagtata | ctccaacaa | aatattttct | aaaagtcac | aacattatac | taccactgag | 11820 |
| attgatctaa | atgacattat | gcaaaatata | gaaccaactt | accctcatgg | attaagagtt | 11880 |
| gtttatgaaa | gtctaccttt | ttataaagca | gaaaaaatag | ttaatcttat | atcaggaaca | 11940 |
| aaatccataa | ctaataatac | tgaaaaaaca | tcagcaatat | atacaactga | tattaatagg | 12000 |
| gctactgata | tgatgaggaa | aaatataact | ttacttataa | ggatacttcc | actagattgt | 12060 |
| aacaaagaca | aaagagagtt | attaagttta | gaaaatctta | gtataactga | attaagcaag | 12120 |
| tatgtaagag | aaagatcttg | gtcattatcc | aatatagtag | gagtaacatc | gccaaagtatt | 12180 |
| atgttcacaa | tggacattaa | atatacaact | agcactatag | ccagtgggat | aattatagaa | 12240 |
| aaatataatg | ttaatagtgt | aactcgtggt | gaaagaggac | ctactaagcc | atgggtaggt | 12300 |
| tcattctacg | aggagaaaaa | aacaatgcca | gtgtacaata | gacaagtttt | aacaaaaaag | 12360 |
| caagagacc | aaatagattt | attagcaaaa | ttagactggg | tatatgcac | catagacaac | 12420 |
| aaagatgaat | tcattggaaga | actgagtact | ggaacacttg | gactgtcata | tgaaaaagcc | 12480 |
| aaaaagtgtg | ttccacaata | tctaagtgtc | aattatttac | accgtttaac | agtcagtagt | 12540 |
| agaccatgtg | aattccctgc | atcaatacca | gcttatagaa | caacaaatta | tcatttcgat | 12600 |
| actagtccta | tcaatcatgt | attaacagaa | aagtatggag | atgaagatat | cgacattgtg | 12660 |
| tttcaaaatt | gcataagttt | tggtcttagc | ctgatgtcgg | ttgtggaaca | attcacaac | 12720 |
| atatgtccta | atagaattat | tctcataccg | aagctgaatg | agatacatct | gatgaaacct | 12780 |
| cctatattta | caggagatgt | tgatatcatc | aagttgaagc | aagtgatata | aaaacagcat | 12840 |
| atgttcctac | cagataaaat | aagttaaac | caatatgtag | aattattcct | aagtaacaaa | 12900 |
| gcacttaaat | ctggatctaa | catcaattct | aatttaatat | tagtacataa | aatgtctgat | 12960 |
| tattttcata | atgcttatat | tttaagtact | aatttagctg | gacattggat | tctaattatt | 13020 |
| caacttatga | aagattcaaa | aggatttttt | gaaaaagatt | ggggagaggg | gtacataact | 13080 |
| gatcatatgt | tcattaattt | gaatgttttc | tttaatgctt | ataagactta | tttgctatgt | 13140 |
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| tggtgttttg | agttaataga | cagtagctac | tggaaatcta | tgtctaaagt | tttcctagaa | 13260 |
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| cacagtttta | agttgtggtt | tttaaaacgc | cttaataatg | ctaaatttac | cgatgcctt | 13380 |
| tgggtgtgta | acatagatta | tcaccaaca | catatgaaag | ctatattatc | ttacatagat | 13440 |
| ttagttagaa | tggggttaat | aaatgtagat | aaattaacca | ttaaaaataa | aaacaaattc | 13500 |
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| catttgctaa | caaaaacaa | aagaattgct | aattcagaat | tagaagataa | ttataacaaa | 13620 |

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| | | | | | | |
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| ctatatcacc | caaccccgaga | aactttagaa | aatatatcat | taattcctgt | taaaagtaat | 13680 |
| aatagtaaca | aacctaatt | ttgtataagt | ggaataaccg | aatctataat | gatgtcaaca | 13740 |
| ttctctaata | aatgcatat | taaatcttcc | actgttacca | caagattcaa | ttatagcaaa | 13800 |
| caagacttgt | acaatttatt | tccaaatggt | gtgatagaca | ggattataga | tcattcaggt | 13860 |
| aatacagcaa | aatctaacca | actttacatc | accacttcac | atcagacatc | tttagtaagg | 13920 |
| aatagtgcatt | cactttattg | catgcttcct | tggcatcatg | tcaatagatt | taactttgta | 13980 |
| tttagttcca | caggatgcaa | gatcagtata | gagtatat | taaaagatct | taagattaag | 14040 |
| gaccccgatt | gtatagcatt | cataggtgaa | ggagctggta | acttattatt | acgtacggta | 14100 |
| gtagaacttc | atccagacat | aagatacatt | tacagaagtt | taaaagattg | caatgatcat | 14160 |
| agtttaccta | ttgaattttc | aagattat | aacgggcata | taaacataga | ttatgggtgag | 14220 |
| aatttaacca | ttcctgctac | agatgcaact | aataacattc | attggtctta | tttacatata | 14280 |
| aaatttgcag | aacctattag | catctttgtc | tgcgatgctg | aattacctgt | tacagccaat | 14340 |
| tggagtaaaa | ttataattga | atggagtaag | catgtaagaa | agtgaagta | ctgttcttct | 14400 |
| gtaaatagat | gcattttaat | cgcaaatat | catgctcaag | atgatattga | tttcaaatta | 14460 |
| gataacatta | ctatatataa | aacttacgtg | tgcctaggtg | gcaagttaaa | aggatctgaa | 14520 |
| gtttacttag | tccttacaat | aggccctgca | aatatacttc | ctgtttttga | tgtgtgcaa | 14580 |
| aatgctaaat | tgattttttc | aagaactaaa | aatttcatta | tgccataaaa | aactgacaag | 14640 |
| gaatctatcg | atgcaaatat | taaaagctta | atacctttcc | ttgtttacc | tataacaaaa | 14700 |
| aaaggaatta | agacttcatt | gtcaaaattg | aagagtgtag | ttaatgggga | tatattatca | 14760 |
| tattctatag | ctggacgtaa | tgaagtattc | agcaacaagc | ttataaacca | caagcatatg | 14820 |
| aatatcctaa | aatggctaga | tcatgtttta | aattttagat | cagctgaact | taattacaat | 14880 |
| cattttatata | tgatagagtc | cacatatcct | tacttaagtg | aattgttaaa | tagtttaaca | 14940 |
| accaatgagc | tcaagaaact | gattaaaata | acaggtagtg | tactatacaa | ccttcccaac | 15000 |
| gaacagtaac | ttaaaatata | attaacaagt | ttggtaaaat | ttagatgcta | acacatcatt | 15060 |
| atattatagt | tattaaaaaa | tatgcaaact | tttcaataat | ttagcttact | gattccaaaa | 15120 |
| ttatcatttt | atttttaag | ggttgaataa | aagtctaaaa | ctaacaatga | tacatgtgca | 15180 |
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<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Positive-sense M gene fragment

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<210> 4
 <211> 31
 <212> DNA
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<220>
 <223> Negative-sense M gene fragment

<400> 4
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<210> 5
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Positive-sense primer upstream of SH gene

<400> 5
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<210> 6
 <211> 20
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<220>
 <223> Negative-sense primer downstream of SH gene

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<220>
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<400> 7
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<210> 8
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<220>
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<210> 9
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<223> Forward PCR primer for NS1 gene deletion

<400> 9
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28

<210> 10
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<220>
<223> Reverse PCR primer for NS1 gene deletion

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32

<210> 11
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<212> DNA
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<223> Forward PCR primer for NS2 gene deletion

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<210> 12
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse PCR primer for NS2 gene deletion

<400> 12
gttttatatt aactaatggt gttagtg
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<210> 13
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward PCR primer for ablation of G gene start site

<400> 13
ttataattgc agccatcata ttcatagcct cgg
33

<210> 14
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<212> DNA
 <213> Artificial Sequence
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<210> 15
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 <212> DNA
 <213> Artificial Sequence
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 <223> Positive-sense primer for intergenic region preceding the G gene
 <400> 15
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<210> 16
 <211> 51
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Negative-sense primer for intergenic region downstream of F gene
 <400> 16
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<210> 17
 <211> 13
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: G gene-end signal
 <400> 17
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<210> 18
 <211> 48
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Positive-sense primer with G gene-end and F gene-start signals
 <400> 18
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<210> 19
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 <212> DNA
 <213> Artificial Sequence

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<220>
 <223> Negative-sense primer with G gene-end and F gene-start signals
 <400> 19
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<210> 20
 <211> 12
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: F gene-end signal of RSV A2
 <400> 20
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<210> 21
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 <223> G gene-end signal of RSV A2
 <400> 21
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<210> 22
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 <220>
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<220>
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 <222> (5)..(5)
 <223> n, if present, is c
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<210> 24
 <211> 51

<212> RNA
 <213> Artificial Sequence
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<210> 28
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<210> 29

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<211> 13
<212> DNA
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<220>
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<400> 29
agttaatata aaa 13

<210> 30
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<220>
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<400> 30
agttaataaa aaa 13

<210> 31
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<212> DNA
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agtaatttaa aa 12

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<211> 13
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<220>
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agttaataaa aaa 13

<210> 33
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<222> (5)..(5)
 <223> Xaa is Ile or Val

<400> 33

Ile Leu Ala Xaa Xaa Ile Ser Thr Ser
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<220>
 <223> Synthetic oligonucleotide

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 <223> Synthetic oligonucleotide

<400> 35
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<210> 37
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<220>
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<400> 37
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 aaataa 66

<210> 38
 <211> 11
 <212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 38

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| Ser | Asn | Ser | Thr | Gln | Asn | Thr | Gln | Ser | His | Ala |
| 1 | | | | 5 | | | | | 10 | |